CLAIMS

We claim:

1. A method of detecting a first target sequence comprising a poly(A) sequence in a sample comprising:

a) hybridizing a first probe to said target sequence to form a first hybridization complex, said first probe comprising:

i) an upstream universal priming site (UUP);

ii) an adapter sequence;

iii) a first target-specific sequence; and

iv) a downstream universal priming site (DUP);

wherein said poly(A) sequence remains single-stranded;

b) contacting said first hybridization complex with a support comprising a poly(T) sequence, such that said poly(A) sequence hybridizes with said poly(T) sequence;

c) removing unhybridized first probe sequences;

d) denaturing said first hybridization complex;

e) amplifying said first probe to generate a plurality of amplicons;

f) contacting said amplicons with an array of capture probes to form assay complexes; and

g) detecting said assay complexes.

2. A method according to claim 1 wherein said first probe comprises a label.

3. A method according to claim 2 wherein said label is a primary label.

4. A method according to claim 3 wherein said primary label is a fluorescent label.

5. A method according to claim 2 wherein said label is a secondary label.

6. A method according to claim 2 wherein said label is biotin.

7. A method of detecting a first target sequence comprising a first target domain, a second adjacent target domain and a poly(A) sequence, said method comprising:

a) hybridizing a first probe comprising:

i) an upstream universal priming site (UUP); and

ii) a first target-specific sequence substantially complementary to said first target domain;

to said first target domain;

b) hybridizing a second probe comprising:

iii) a second target-specific sequence substantially complementary to said second target domain;

iv) a downstream universal priming site (DUP);

wherein at least one of said first and second probes comprises at least a first adapter sequence, said poly(A) sequence remains single-stranded, and said target sequence and said first and second probes form a ligation complex;

c) contacting said ligation complex with a ligase to form a ligated complex;

d) contacting said ligated complex with a support comprising a poly(T) sequence, such that said poly(A) sequence hybridizes with said poly(T) sequence;

e) removing unhybridized first and second probe sequences;

f) denaturing said ligation complex;

g) amplifying the ligated first and second probes to generate a plurality of amplicons;

h) contacting said amplicons with an array of capture probes to form assay complexes; and

i) detecting said assay complexes.

8. A method according to claim 7 wherein said first target domain and said second target domain are directly adjacent.

9. A method according to claim 7 wherein said first target domain and said second target domain are separated by at least one base and said method further includes contacting said ligation complex with a polymerase and at least one dNTP.

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10. A method according to claim 7 wherein one of said first and second probes comprises a label.

11. A method according to claim 10 wherein said label is a primary label.

12. A method according to claim 11 wherein said label is a fluorescent label.

13. A method according to claim 10 wherein said label is a secondary label.

14. A method according to claim 13 wherein said secondary label is biotin.

15. A method according to claim 1 or 7 wherein said amplifying is done by:

a) hybridizing a first universal primer to said UUP;

b) providing a polymerase and dNTPs such that said first universal primer is extended;

c) hybridizing a second universal primer to said DUP;

d) providing a polymerase and dNTPs such that said second universal primer is extended; and

e) repeating steps a) through d).

16. A method according to claim 1 or 7 wherein said array comprises:

a) a substrate with a patterned surface comprising discrete sites; and

b) a population of microspheres comprising at least a first subpopulation comprising a first capture probe and a second subpopulation comprising a second capture probe.

17. A method according to claim 16 wherein said discrete sites comprise wells.

18. A method according to claim 16 wherein said substrate comprises a fiber optic bundle.

19. A method according to claim 1 or 7 wherein said support comprising a poly(T) sequence comprises magnetic beads.